



# Discovery of a reproducing population of the Mindo Glassfrog, *Nymphargus balionotus* (Duellman, 1981), at the Río Manduriacu Reserve, Ecuador, with a literature review and comments on its natural history, distribution, and conservation status

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**Abstract.**—The recently established Río Manduriacu Reserve, located on the Andean slopes in northwestern Ecuador, has proven to be a site of high conservation importance for amphibians. It harbors a range of threatened species, including the only known population of the Critically Endangered Tandayapa Andes Toad, *Rhaebo olallai*, as well as those of two recently described frog species. Herein, the conservation value of the reserve is further bolstered with the discovery of a new population of the rare and enigmatic glassfrog, *Nymphargus balionotus*. Prior to this finding, and aside from a single record in 2005, no observations of this species have been reported from throughout its narrow range within NW Ecuador and western Colombia since 1984. This marks the sixth locality reported for *N. balionotus*, the third site to yield more than one individual, and the first documentation from within a protected area. Also presented are the first observations of amplexus, egg masses, and the metamorphic life stage. Published literature pertaining to *N. balionotus* is difficult to follow, especially reports on Colombian material; therefore, a comprehensive review of the literature and discussion of previously unpublished details regarding Colombian records is provided. The population at the Río Manduriacu Reserve is currently the only known extant population of *N. balionotus*, and its immediate future is uncertain due to pressure from a mining company that is currently prospecting in-and-around the reserve.

**Keywords.** Amphibian conservation, *Cochranella balionota*, Endangered, Imbabura, rediscovery, Threatened glassfrog, threatened by mining

**Resumen.**—La nueva Río Manduriacu Reserve, localizada en las laderas andinas del noroeste de Ecuador, es un lugar de gran importancia para la conservación de anfibios amenazados, ya que alberga una gran cantidad de especies amenazadas, incluyendo la única población conocida del Andinosapo de Olalla, *Rhaebo olallai*, En Peligro Crítico, además de dos especies de ranas recientemente descritas. Aquí, la importancia de conservar la reserva se ve bien reforzada con el descubrimiento de una nueva población de la rara y enigmática rana de cristal, *Nymphargus balionotus*. Previo a esto, excepto por un único registro en 2005, desde 1984 no se habían reportado observaciones en todo su estrecho rango en el Noroeste de Ecuador y Oeste de Colombia. Esto supone la sexta localidad reportada para *N. balionotus*, la tercera localidad en la que se ha encontrado más de un individuo y el primer registro dentro de un área protegida. También presentamos el primer registro de un amplexus, la primera puesta de huevos y el primer individuo metamorfo. La literatura publicada sobre *N. balionotus* es difícil de seguir, especialmente los trabajos basados en material colombiano; por lo tanto, proporcionamos una revisión exhaustiva de la literatura y discutimos detalles previamente no publicados sobre los registros colombianos. Sin embargo, la falta de información sobre tres de las localidades conocidas ha dificultado la reevaluación de su estado de amenaza. La población de la Reserva Río Manduriacu es actualmente la única población existente conocida de *N. balionotus* y su futuro próximo es incierto debido a la presión de una compañía minera que actualmente está explorando la reserva.

**Palabras clave.** Conservación de anfibios, *Cochranella balionota*, rana de vidrio En Peligro, Imbabura, redescubrimiento, rana de vidrio amenazada, amenazado por la minería

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## Introduction

The Mindo Glassfrog, *Nymphargus balionotus* (Duellman 1981), is a poorly known glassfrog from the western versant of the Cordillera Occidental of the Andes in Ecuador and Colombia. This relatively small and striking species was described primarily based on a series of 13 males collected along a small, cascading stream at a site just northeast of Mindo, Ecuador, in April 1975 (1,540 m; Duellman 1981). Also referenced in the description was a single specimen (KU 145084) collected in March 1938 from a site ~330 km north of the type locality, near El Tambo, Cauca, Colombia. Unfortunately, only limited information is associated with the latter specimen (see VertNet 2019).

In the time since *N. balionotus* was described, new information has been scant; search efforts in ensuing years have yielded just two individuals in Ecuador, and one population in Colombia. The first of the Ecuadorian specimens was collected in November 1984 at a site near Cabeceras del Río Baboso, in western Carchi province (DHMECN 0865; ca. 1,400 m; 0°52'59"N, 78°27'W; Cisneros-Heredia and Yanez-Muñoz 2007), and the second was collected from an area of primary lower montane forest NE of La Maná, Río Lomapi, Cotopaxi, in August 2005 (FHGO 5564; 1,283 m; 0°48'55"S, 79°05'01"W; Jorge Valencia, pers. comm.). Some of the only known color photographs of live *N. balionotus* specimens are of the latter individual, and can be seen in Guayasamin and Frenkel (2018; photos by Martín Bustamante). The Colombian population was recorded at Campamento Chancos, Vereda Campo Alegre, Municipio de Restrepo, Valle del Cauca (Ruiz-Carranza et al. 1996; Lynch and Suarez-Mayorga 2004), where nine specimens were collected (Lynch and Ruiz-Carranza 1996). These records were observed at a considerably lower elevation (460 m; Lynch and Suarez-Mayorga 2004), and extended the known range of *N. balionotus* ca. 160 km north from the single record from the Cauca Department. Similar to the specimen from Cauca, few details have been published on the material or the site of collection (see **Discussion**). Nonetheless, this remains the only site other than the type locality where more than one individual of *N. balionotus* has been recorded.

Until recently, the phylogenetic position of *N. balionotus* has been unclear. Duellman (1981) originally placed the taxon in the genus *Centrolenella*, which was subsequently designated to *Cochranella* by Ruiz-Carranza and Lynch (1991) based on the absence of

humeral spines in males from specimens collected at Campamento Chancos, Valle del Cauca. However, Cisneros-Heredia and McDiarmid (2006) verified the presence of humeral spines in the material described by Duellman (1981), thus combining the taxon with *Centrolene*, and the Colombian material reported by Lynch and Ruiz-Carranza (1996) was therefore presumed to be of a distinct lineage (Cisneros-Heredia and McDiarmid 2006). Later, in a monographic revision of the glassfrog family, Centrolenidae, Guayasamin et al. (2009) underscored the difficulty of substantively designating the taxon at the generic level, and regarded the taxon as *incertae sedis* within the subfamily Centroleninae. Finally, after incorporating mitochondrial sequences into a phylogenetic analysis of the family, *Centrolenella balionota* was recovered within the genus *Nymphargus*, and as sister to the recently described, and Critically Endangered, *N. manduriacu* (Guayasamin et al. 2019).

Despite numerous efforts, and even the presence of seemingly appropriate habitat at the known localities in Ecuador, recent attempts to find *N. balionotus* within its historical range and nearby localities have failed (Cisneros-Heredia and Yanez-Munoz 2007; Ron et al. 2011; Arteaga et al. 2013; IUCN SSC Specialist Group 2020). In Colombia, the most recent records are those from northern Valle del Cauca Department, and date back to 1984 (see **Discussion**; IUCN SSC Specialist Group 2020). The single specimen collected in 2005 from Río Lomapi, Cotopaxi Province, Ecuador, is the last reported observation of this enigmatic glassfrog. Herein, the discovery of a reproducing population of *N. balionotus* is reported from the recently established Río Manduriacu Reserve (RMR), Imbabura, Ecuador. The data from RMR include the first records of metamorphs and egg masses of the species. Aspects regarding its natural history, distribution, and conservation status are discussed, and detailed color photographs of various life stages and reproductive behavior are presented. Lastly, a comprehensive review of the literature pertaining to *N. balionotus* is provided and new details concerning Colombian material that were uncovered in the process are discussed.

## Materials and Methods

**Study site.** Fieldwork was carried out at the Río Manduriacu Reserve (RMR), located on the Pacific Andean slopes in western Imbabura, Ecuador (1,100–

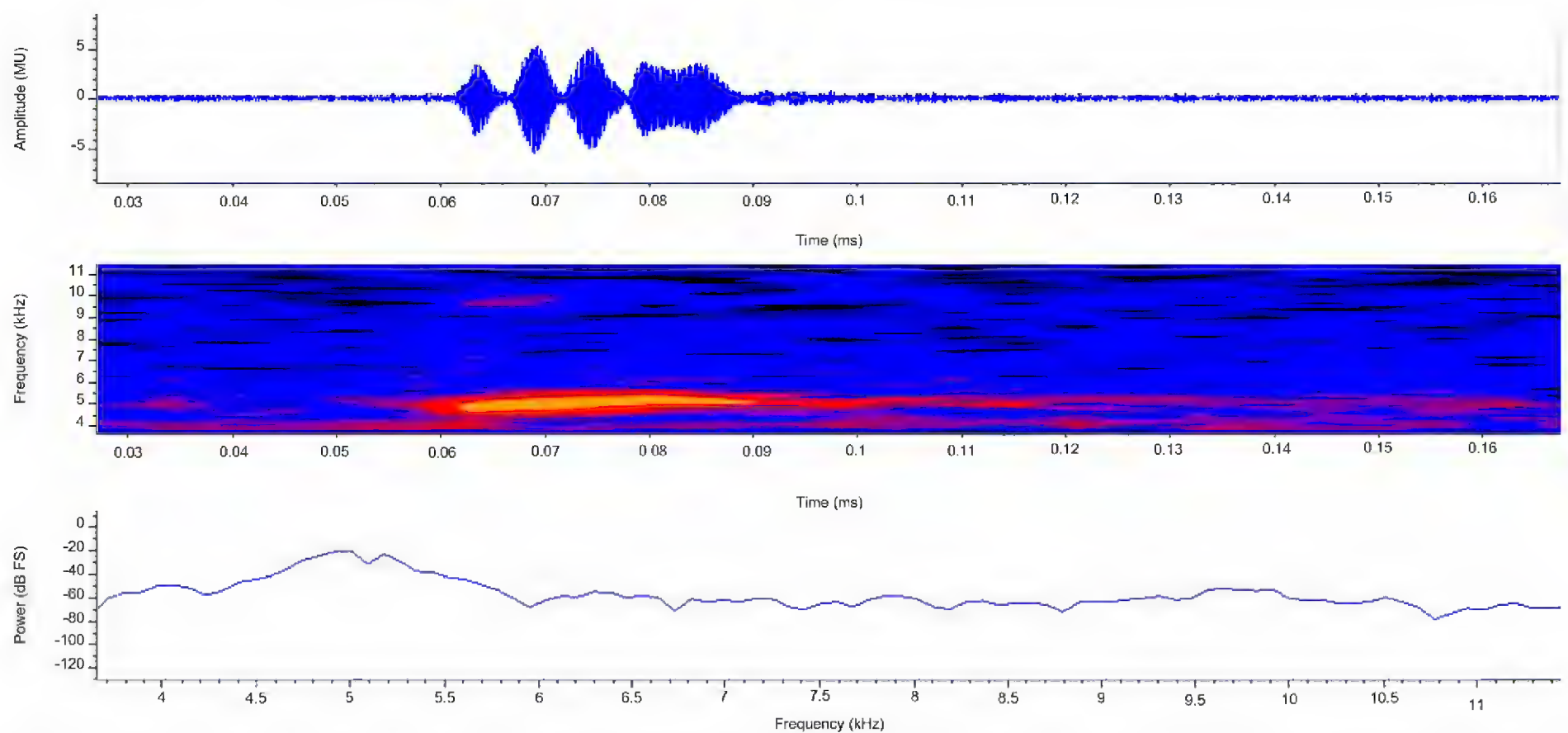


**Fig. 1.** Distribution map of known localities for *Nymphargus balionotus*. Information tags to the left summarize data reported for each locality, including (in order): name of collection site, department/province, number of specimens reported, elevation, and year specimens were collected or observed. Blue circle represents the Río Manduriacu Reserve; red circle marks the type locality; yellow and gray circles represent remaining localities reported in the literature, with the gray (Campamento Chancos) population believed to be a distinct lineage by Cisneros-Heredia and McDiarmid (2006). Note: the “El Tambo” locality in the department of Cauca, Colombia, has been placed as accurately as possible based on available information (see **Discussion**).

2,000 m; Fig. 1). The RMR is a privately protected area that consists of ca. 600 ha managed by Fundación EcoMinga. Habitat at RMR consists of primary and mature secondary lower montane and cloud forest habitat. The reserve is situated along the east-facing slope on the west side of a north-south oriented river canyon; the Río Manduriacu bisects the canyon, flowing from north to south. Most of the habitat within RMR is undisturbed, with only minor disturbances at the lower reaches of the reserve from selective timber extraction, a clearing for a cabin used by researchers and the EcoMinga reserve guards, as well as a limited number of recently created small-scale clearings resulting from mining prospecting. Further details of the study site are provided by Lynch et al. (2014) and Guayasamin et al. (2019).

**Sampling.** Sampling efforts at RMR follow the time frames outlined in Guayasamin et al. (2019), as well as two additional sampling efforts from 28 February to 13 March 2019 (by RJM, SK, ST, JC, José Maria Loaiza, Rolando Peña, and two assistants) and 23 November to 17 December 2019 (by RJM, ST, JC, José Maria Loaiza, Rolando Peña, and one assistant). The survey period from 8–11 April outlined in Guayasamin et al. (2019)

is amended here from 2018 to 2017. Visual encounter surveys were the primary sampling method. Surveys were conducted along transects of various lengths within primary, secondary, and riparian forest, and along streams of various sizes. For smaller streams that were densely vegetated and too narrow to transect, general area searches of the stream were performed. Call surveys were also used, where calls of glassfrogs were specifically targeted in streams, especially during and after mild rain events. Sampling localities were primarily within the RMR, however forest trails and streams outside the reserve boundaries to the south, and on the opposite/east side of the Río Manduriacu, were also surveyed. Surveys were conducted between 1900 and 0200 h. Data collection included the following parameters: relative humidity, ambient temperature (°C), date, time of observation, geographic coordinates, gender, age class (adult; subadult; juv/metamorph; tadpole; egg mass), behavior (if any), snout-vent length (SVL, mm; only for captured individuals), perch height (when applicable), and perch diameter (when applicable). Climatic data were collected using a Kestrel 3500 Weather Meter, geographic coordinates with a Garmin GPSmap 62s handheld unit, and SVL with dial calipers.



**Fig. 2.** Audio spectrogram (top), oscillogram (middle), and power spectrum (bottom) graphs of a single male *Nymphargus balionotus* advertisement call.

**Bioacoustics.** Call analysis is based on two recordings of advertisement calls from a single male *N. balionotus* obtained by SJT on 30 November 2019 at 1930 h during light rain and ambient air temperature measuring 21.2 °C (Fig. 2). The two recordings were made with a Rode VideoMic microphone connected to a Sony PCM-M10 Recorder, with a sampling rate of 44.1 kHz, 24-bit resolution and recorded in .wav format. The microphone was placed approximately 2.5 m and 1.5 m from the calling male during the 1<sup>st</sup> and 2<sup>nd</sup> recordings, respectively; both calls were combined into one file, unaltered, and deposited at the Cornell University Macaulay Library (catalog number: ML237497; <https://macaulaylibrary.org/asset/237497>). Outlier properties within the calls were removed from the respective measurement results. Each call was analyzed for both temporal and spectral domains (Raven Pro 1.6.1; Cornell Lab of Ornithology, Ithaca, New York, USA), following the recommendations of Köhler et al. (2017).

Adobe Audition (version 13.0.1.35) sound removal process was used to generate a recording to facilitate the measurement of temporal variables. Sound removal was applied with the following settings: sound model complexity 60, sound refinement passes 150, content complexity 60, and content refinement passes 150. For the first call recording, the sound model was trained and applied with timeline segment 2:50–3:00 min. For the second call recording, the sound model was trained and applied with timeline segment 2.4–5.7 s. Frequencies for both recordings were filtered out below 4,350 Hz (below the fundamental harmonic) due to significant background noise using the “Band Filter” function after viewing the spectrogram in Raven without significantly modifying call properties.

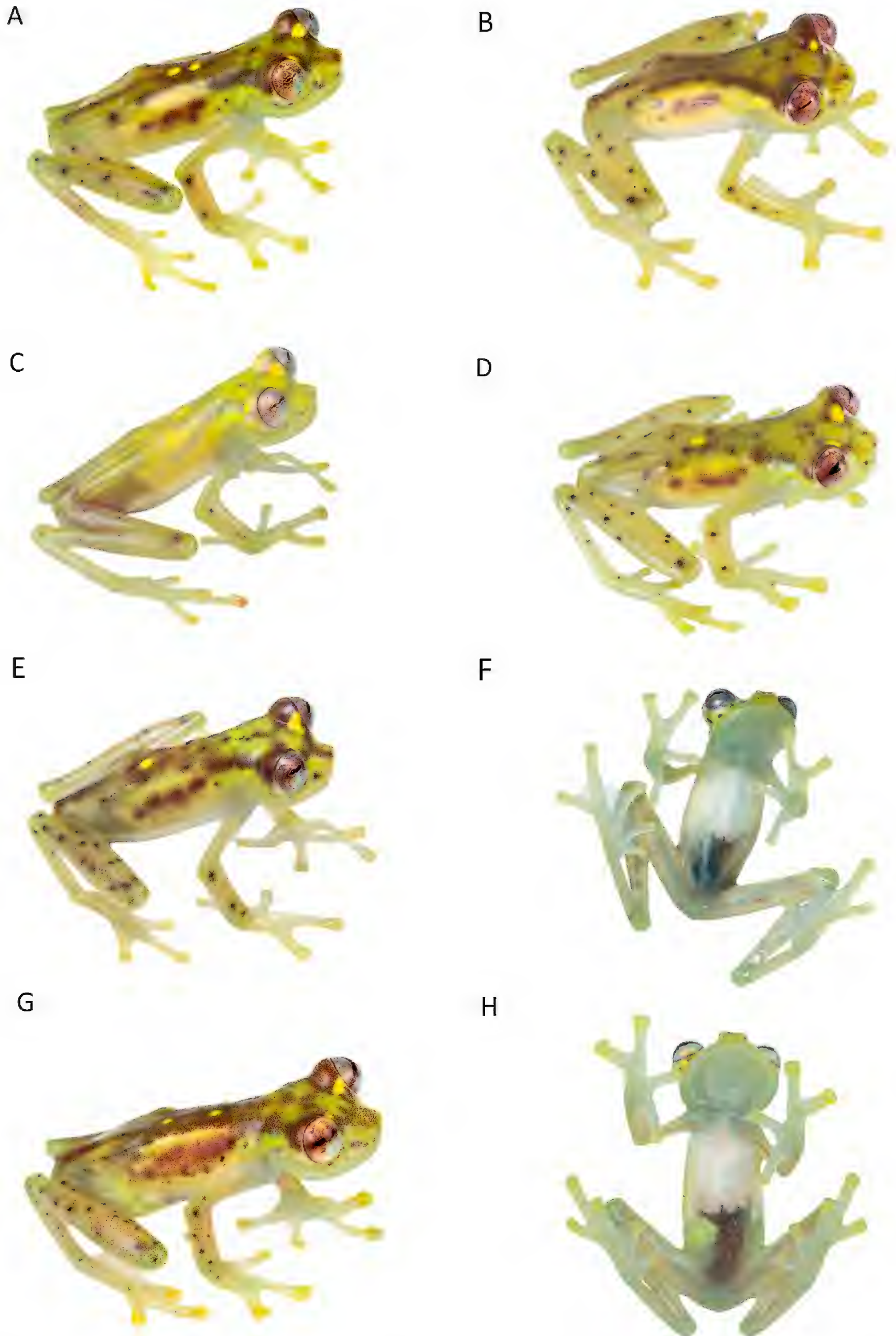
Call duration was defined as the length of a note. Call period was the time interval from the beginning of one

note to the beginning of the next note. Call repetition rate was the inverse of call period. The number of pulses was the number of pulses in a note. Pulse rate was calculated as the number of pulses in a note divided by call duration. Call bandwidth was measured using the “Freq 5%” and “Freq 95%” measurement functions in Raven Pro 1.6.1. Dominant frequency was defined as the frequency with the most energy. Spectrogram configuration was set at a Hann window of 512-sample window size, 256-sample hop size with 50% frame overlap, and 86.1-Hz frequency grid spacing.

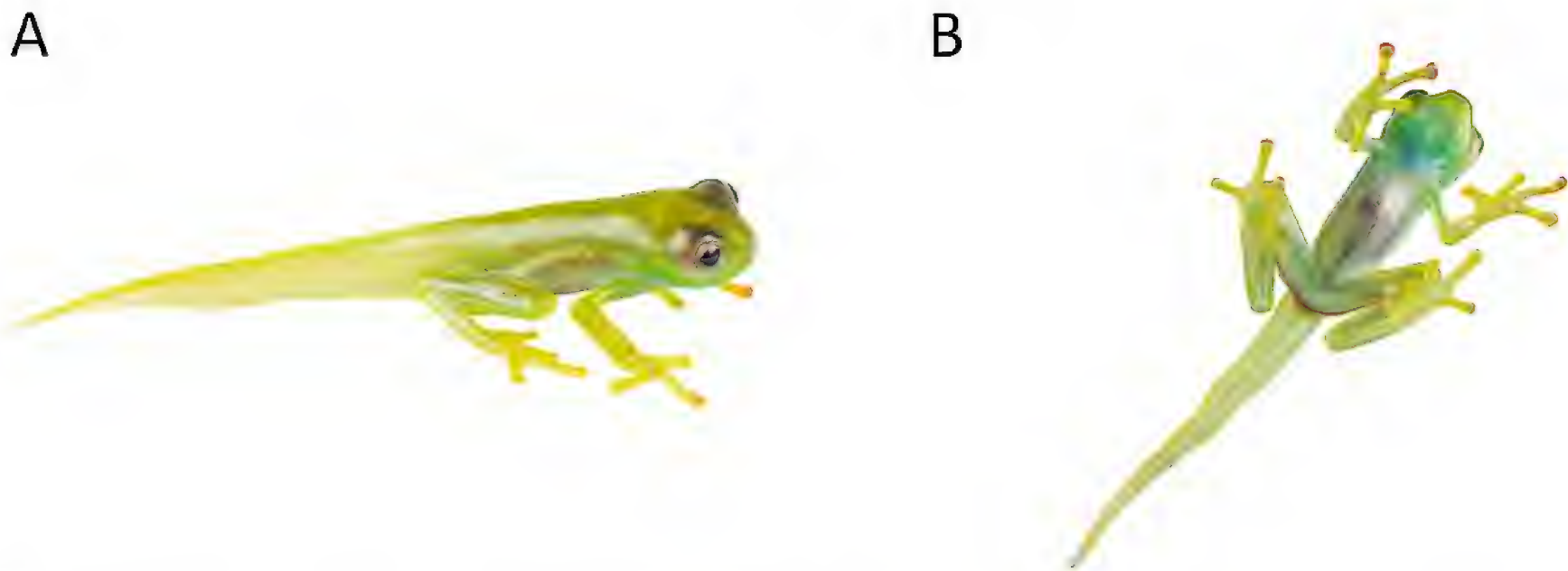
**Specimen collection and ethics statement.** Collected specimens were euthanized using benzocaine and were fixed and preserved in 70% ethanol. Muscle and liver samples were preserved in 96% ethanol. All specimens collected from RMR were deposited at the Museo de Zoología of the Universidad San Francisco de Quito (ZSFQ). Specimens were collected under permits N°018-2017-IC-FAU-DNB/MAE and N°019-2018-IC-FAU-DNB/MAE and authorized by the Ministerio del Ambiente del Ecuador. Specimen transport was approved through the Guia de Movilización de Especímenes de Fauna Silvestres, emitted on 2 March 2018. The study was carried out in accordance with the guidelines for use of live amphibians and reptiles in field and lab research (Beaupre et al. 2004), compiled by the American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists’ League (HL), and the Society for the Study of Amphibians and Reptiles (SSAR).

## Results

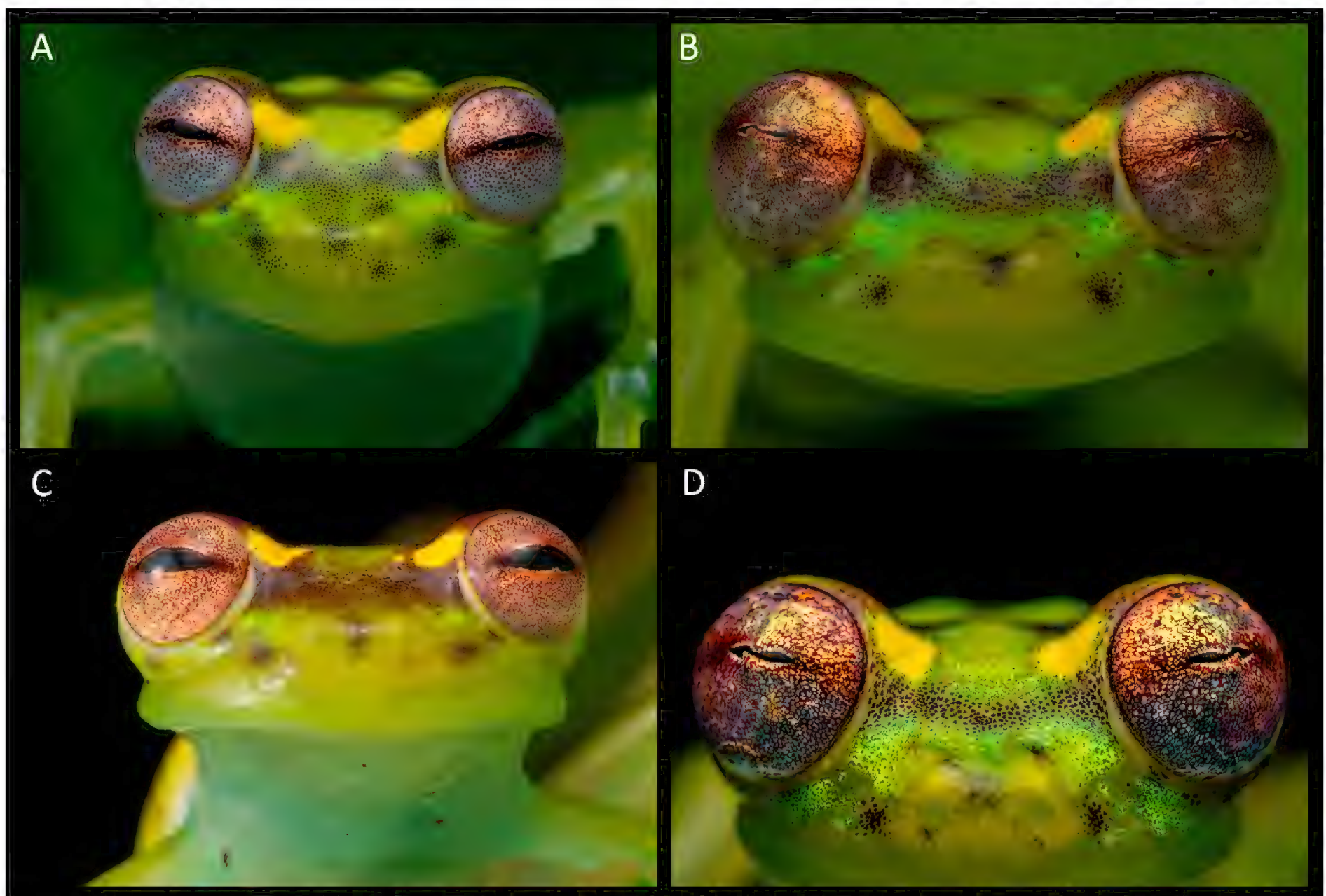
The data reported here are from a previously undocumented population of *Nymphargus balionotus* from western Imbabura Province, Ecuador, at the Río



**Fig. 3.** Pattern and color variation in adult *Nymphargus balionotus*. (A) Male, uncollected; (B) Male, ZSFQ 0531; (C) Male, uncollected; (D) Male, uncollected; (E–F) Male, ZSFQ 0532; (G–H) Gravid female, uncollected. Photos by Ross J. Maynard.



**Fig. 4.** Metamorphic life stage of *Nymphargus balionotus*; dorsal (A) and ventral (B) views (ZSFQ 3895). Photos by Ross J. Maynard.



**Fig 5.** Iris variation in *Nymphargus balionotus*. Photos by Jaime Culebras (A–C) and Scott Trageser (D).

Manduriacu Reserve (0°31'N, 78°51'W; Fig. 1). A total of 39 observations were made during the months of March (2019), November (2019), and December (2019); no individuals were observed during sampling efforts prior to 2018. All individuals were observed at night from 1950–2355 h, at elevations spanning 1,116–1,285 m. Records consisted of five females, 28 males, two subadults, two metamorphs, and two egg masses (Figs. 3–6). One of the two egg masses contained 17 eggs (Fig. 6). Only one adult pair was observed in amplexus on 7 March 2019 (Fig. 6). Five specimens were collected and deposited with tissues (ZSFQ 0531–33, 0536, 3895).

**Habitat and natural history.** Of a total of 17 streams surveyed within ( $n = 13$ ) and outside of ( $n = 4$ ) RMR, four streams were occupied by *N. balionotus*. Three of the four occupied streams were within the boundaries of RMR, and the one outside of the reserve was on the slope east of the Río Manduriacu. The occupied streams within the reserve are roughly parallel to one another from south to north, have a combined distance between the streams of 0.5 km, and are situated within primary lower montane forest at the points of observation. All records within RMR ( $n = 29$ ) were within 1,231–1,285 m asl. The greatest distance between observations in the reserve



**Fig. 6.** *Nymphargus balionotus* breeding behavior. (A) Calling male; (B) Male and female in amplexus; (C) Egg mass deposited on the bottom surface on a leaf. Photos by Ross J. Maynard (A) and Jaime Culebras (B–C).

was 490 m. Observations at the stream outside of the reserve ( $n = 10$ ) were recorded between 1,116–1,135 m asl and were also from within an area with mature forest. Unlike the habitat within RMR, cattle pasture is quickly encroaching on this segment of forest from the north and south of the observation points. A barbed-wire fence has been erected running parallel to a portion of the ca. 100 m section of stream which was surveyed, indicating that cattle pasture will potentially replace this patch of forest in the near future.

All four occupied streams were moderate- to fast-flowing, cascading, and characterized by the presence of various sized boulders. The stream outside the boundaries of RMR, and one of the three within RMR, were 1–3 m

in width; and the other two streams within RMR were wider (3–6 m width) and faster flowing. Fewer streams were located on the east side of the Río Manduriacu ( $n = 3$ ), as navigating the forest was difficult due to a lack of established trails aside from cattle paths leading to the open patches of pasture.

Most of the *N. balionotus* observations (90%) were recorded in groups of three or more individuals in close proximity to one another, either within the same vegetation (i.e., leaves of the same tree or cluster of vegetation) or within a specific area of a stream (i.e., a single 1–5 m stretch). Four such “hotspots” were recorded, three of which were located in the two narrower of the four occupied streams; the 4<sup>th</sup> hotspot was in one of the two wider streams, where a large tree had fallen across it. Two hotspots were within RMR and yielded observations of *N. balionotus* in February 2018, and March, November, and December 2019. The two hotspots in the narrow stream across from RMR were separated from one another by ca. 90 m and collectively consisted of nine calling males and one metamorph. This stream was surveyed just once in March 2019. Individuals found at locations away from the hotspots consisted of three males calling in isolation (i.e., no other males were seen or heard nearby), and a female that was observed in close proximity to one of the isolated males.

Males were observed calling while perched on the top side of leaves (Fig. 6); perches were directly above, or immediately adjacent to, the water. Perch heights ranged from 1–5 m. On multiple occasions, the same male was found to be calling within the same group of leaves (or leaf) during consecutive nights. Males called regardless of whether it was raining, however rain events occurred on all days when observations were made; males called more often during light or steady rain and when relative humidity was above 90%. Egg masses were deposited on the underside of leaves of an unidentified fern and an unidentified tree species (Fig. 6).

**Call analysis.** Fifteen calls were recorded in two nearly contiguous recordings. Four of these calls exhibited incongruent properties, and one call overlapped with the advertisement call of *Espadarana prosoblepon* for which the call duration could not be determined. These five calls were excluded from all analyses. The four calls exhibiting incongruent properties are suspected to have resulted from insufficient call motivation due to the recorded male moving to adjacent leaves between those calls. The remaining 10 calls have relatively consistent properties and are representative of high call motivation. This notion was supported by a visual comparison with the spectrogram from a single call recording of a different male also considered to be produced with strong call motivation (recorded on iPhone by JC).

Each call is a high-pitched chirp that consists of a single pulsatile note (Fig. 2). Call duration was measured as 105–130 ms ( $\bar{x} = 114 \pm 1$ ;  $n = 10$ ) with an intercall

duration of 20.9–84.2 s ( $\bar{x} = 41.4 \pm 22.7$ ;  $n = 10$ ). Call period was measured as 21.04–84.29 s ( $\bar{x} = 41.43 \pm 22.69$ ;  $n = 10$ ). Call repetition rate was measured as 0.012–0.048/s ( $\bar{x} = 0.030 \pm 0.013$ ;  $n = 10$ ). The calls exhibit a parallel frequency band with dominant frequency ranging from 9,733–10,250 Hz ( $\bar{x} = 9967 \pm 197$ ;  $n = 10$ ). The calls generally ( $n = 10$ ) contain a pulsatile note with indistinguishable amplitude modulation present on either end of the call and 2–3 distinctly separated pulses in its center, immediately followed by either 1–2 additional pulses or a fused pulse. Pulses per note ranged from 3–5 ( $\bar{x} = 4.1 \pm 0.6$ ;  $n = 10$ ). Pulse rate varied from 28–46/s ( $\bar{x} = 36 \pm 6$ ;  $n = 10$ ). In each call, there is a slight increase in the dominant frequency with time. Call bandwidth ranged from 4,565 Hz ( $\bar{x} = 4,814 \pm 125$ ;  $n = 10$ ) to 5,426 Hz ( $\bar{x} = 5,359 \pm 57$ ;  $n = 10$ ) with a dominant frequency of 4,737–5,254 Hz ( $\bar{x} = 5,082 \pm 161$ ;  $n = 10$ ).

Among species closely related to *N. balionotus*, the calls of *N. manduriacu* (Guayasamin et al. 2019) and *N. grandisonae* (Hutter et al. 2013) have been described. The call of *N. balionotus* is differentiated mainly by having a lower number of pulses per note, with 3–5 ( $\bar{x} = 4.1 \pm 0.6$ ;  $n = 10$ ) in *N. balionotus*, versus 8–12 ( $\bar{x} = 10.33 \pm 1.366$ ) in *N. manduriacu*; a longer intercall duration of 20.9–84.2 s ( $\bar{x} = 41.4 \pm 22.7$ ;  $n = 10$ ) in *N. balionotus*, versus 3.9–8.6 s ( $\bar{x} = 5.72 \pm 1.82$ ) in *N. manduriacu*; and a higher dominant frequency of 4,737–5,254 Hz ( $\bar{x} = 5,082 \pm 161$ ;  $n = 10$ ) in *N. balionotus* versus 4,052–4,447 Hz ( $\bar{x} = 4,267 \pm 118$ ) in *N. manduriacu* and 3,100–4,048 Hz ( $\bar{x} = 3,587 \pm 189$  Hz;  $n = 417$ ) in *N. grandisonae*. The calls of *N. balionotus* and *N. manduriacu* do not exhibit harmonics, while the call of *N. grandisonae* does exhibit harmonics. *Nymphargus balionotus* contains a parallel frequency band while no parallel frequency bands are exhibited in either *N. manduriacu* or *N. grandisonae*.

**Pattern, color, and size variation.** Adding to the description of color in life by Duellman (1981): dorsum of adults lime-green to metallic-green, with rust or brown-copper stripes from the posterior of the eyes to one- to two-thirds down the length of the dorsum, and a chevron between the anterior interorbital region; prominent rust to brown-copper blotches or moderate to sparse stippling may also be present; few to many dark splotches scattered on dorsal surfaces of the back, arms, and legs; 1–2 bright yellow mid-dorsal spots may or may not be present; a bright yellow spot (or blotch) always present on the upper-eyelids (Figs. 3, 5). Measured adult males had an SVL of 19.0–21.0 mm ( $n = 6$ ) and females 21.7–22.0 mm ( $n = 2$ ); metamorphs measured 11.7 and 15.4 mm. Adults do not appear to demonstrate sexual dimorphism in size or color, however males are easily determined by the presence of a humeral spine (Fig. 3).

**Diversity.** Glassfrog diversity is exceptionally high at RMR, with nine total species documented. Syntopic centrolenid species with *N. balionotus* at streams within

RMR include: *Centrolene peristicta*, *Espadarana prosoblepon*, *Hyalinobatrachium valerioi*, *Nymphargus manduriacu*, and *Sachatamia orejuela*; one species was recorded syntopically on the stream outside the reserve: *Espadarana prosoblepon*. Three other glassfrog species are found at RMR (*Centrolene ballux*, *Cochranella* sp., and *Nymphargus grandisonae*), however these species have only been recorded at the upper reaches of the reserve near the western ridgeline of the canyon. Only *Centrolene peristicta* has been found at both the lower and upper reaches of RMR (i.e., at 1,200–1,800 m).

## Discussion

The records reported here from the Río Manduriacu Reserve represent the first observations of *N. balionotus* in 13 years, as well as the first documentation of a reproducing population in over three decades (IUCN SSC Specialist Group 2020). In fact, the population at RMR is currently the only known extant population of this rare glassfrog, and is the first to range within a protected area. Moreover, these records include repeated same-site observations spanning nearly two years (i.e., February 2018, March 2019, November/December 2019), and comprise the first observations of egg masses and metamorphs of *N. balionotus*, indicating that the population is currently healthy.

**Natural history and distribution at the Río Manduriacu Reserve.** Although members of *Nymphargus* are known to deposit their egg masses on the tops of leaves overhanging water, *N. balionotus* appears to instead utilize the underside of leaves, similar to *Hyalinobatrachium* spp. and some other species (Fig. 3; e.g., *Centrolene antioquiensis*, *C. peristicta*, *C. notosticta*, *Teratohyla spinosa*; Guayasamin et al. 2009; Salgado and Guayasamin 2018). Considering that most of the five glassfrog species syntopic with *N. balionotus* at RMR utilize the January–March wet season for breeding—only *Sachatamia orejuela* has not been observed breeding during this time-frame at RMR—perhaps this character is the result of niche partitioning in this *N. balionotus* population. For example, *Espadarana prosoblepon* and *N. manduriacu*, which are known to deposit egg masses on the tops of leaves, have been observed breeding at similar heights above the water and on vegetation in close proximity to calling males of *N. balionotus* (Guayasamin et al. 2019).

The distribution of *N. balionotus* at RMR is also notable. Despite the moderate size and broad elevational gradient encompassed at RMR, records from within and just outside of the reserve are highly limited in area and elevation, and are restricted to streams within old growth forest. Surprisingly, surveys on a number of other streams at higher and lower elevations, and more interior in the canyon, did not yield additional records. In fact, there were no observations of *N. balionotus* on 76% of



the surveyed streams. Perhaps the species is more widely distributed at RMR, but in areas that we were unable to sample due to the challenging landscape. Furthermore, considering that research trips prior to the February 2018 expedition had a strong emphasis on stream surveys—a result of the rediscovery of the Critically Endangered obligate stream associate, *Rhaebo olallai*—it is odd that *N. balionotus* was not documented during earlier surveys along the same streams in RMR where we now know they occur. Perhaps differences in weather conditions were a contributing factor, as a majority of the current records (85%) correspond to the January–March wet season. The remaining 15% of observations made during late November and December 2019 could also correspond to similar rainfall typical of the wet season, however site-specific weather data at RMR is unfortunately lacking. Continued research at RMR and the ability to collect temporal data across seasons and years is necessary in order to understand the relationship between behavioral patterns of *N. balionotus* with seasonal shifts in weather.

**Literature review, with emphasis on Colombian specimens.** Colombian records of *N. balionotus* are problematic. The first notable issue is the difficulty in identifying where the single specimen from the department of Cauca was actually collected. Information associated with KU 145084 indicate it was collected from El Tambo at an elevation of 800 m (see VertNet 2019); however, elevations within the vicinity of El Tambo are ~1,500 m at the lowest point. Furthermore, El Tambo is situated within the inter-Andean valley as opposed to the western versant of the Cordillera Occidental, where *N. balionotus* is otherwise known to occur. Duellman (1981) presumably realized this issue in referencing a general locality of “La Costa,” while omitting any reference to El Tambo. However, if Duellman intended for La Costa to reference a specific locality, which is unclear, we were unable to identify a location by that name within Cauca. The nearest area corresponding to 800 m elevation and the correct versant of the Cordillera Occidental is situated ca. 30 km west of El Tambo, between Huisito and Cocal, Cauca. We suspect that this is approximately the area in which the specimen was actually collected. Further complicating the issue, “El Tambito” has been erroneously reported as its locality in numerous accounts (Bolivar et al. 2004; Stuart et al. 2008; Guayasamin and Frenkel 2018). Regardless, the site of collection for KU 145084 should be considered imprecise.

Information pertaining to specimens collected from the department of Valle del Cauca is also conflicting and difficult to follow. When Ruiz-Carranza and Lynch (1991) combined the taxon with *Cochranella* due to the absence of humeral spines in males, they contradicted the description of the same character by Duellman (1981). The authors presumably arrived at this conclusion by either analyzing new Colombian material or reassessing the type series, but a justification was never provided.

The answer was subtly revealed five years later when a series of nine previously unreported specimens of *C. balionota* appeared in the list of examined material for a new species of glassfrog described by the same authors (Lynch and Ruiz-Carranza 1996). Nevertheless, the localities of these specimens remained unpublished. The first account to broadly indicate where these specimens were collected, albeit vaguely, was by Ruiz-Carranza et al. (1996), as they reported *C. balionota* from Ecuador up to 4 °N, at 400–800 m, which indeed corresponds to a latitude encompassing much of the Department of Valle del Cauca. That was also the first account to report an elevation other than 800 m from Colombia, providing further evidence of a new locality for the species. Campamento Chancos was not reported as a locality for *C. balionota* (= *N. balionotus*) in Colombia until another eight years later by Lynch and Suarez-Mayorga (2004), which has remained the only peer-reviewed account to do so.

Adding to the perplexing obscurity of the specimens from Campamento Chancos, fundamental data such as who collected the material, the date of collection, a habitat description, behavior, gender, and geographic coordinates of the collection site have all been omitted from the literature, thereby leaving a number of gaps in basic knowledge about these specimens, including the date at which *N. balionotus* was last seen in Colombia. Therefore, instead of largely adding to what is known about the species, published accounts pertaining to these specimens have arguably done more to sow confusion about this population and where the site of collection is located, while complicating the ability to properly evaluate the threatened status of this rare species (IUCN SSC Specialist Group 2020). In fact, the omissions of relevant data and the lack of clarity have led to a number of errors in published accounts of *N. balionotus* by others (Acosta-Galvis 2000; Bolívar et al. 2004; Cisneros-Heredia and Yáñez-Muñoz 2007; Stuart et al. 2008; Guayasamin and Frenkel 2018). Although this circumstance potentially reflects the nature of the specimens themselves, this also is never made clear by authors that have specifically reported on these specimens (i.e., Ruiz-Carranza and Lynch 1991; Lynch and Ruiz-Carranza 1996; Ruiz-Carranza et al. 1996; Lynch and Suarez-Mayorga 2004).

Nevertheless, a lack of information associated with the specimens from Campamento Chancos does not appear to be the case. Campamento Chancos is evidently the type locality for the Red-thighed Thin-toed Frog, *Leptodactylus rhodomerus*, and the type series was collected at this site by John D. Lynch in May and June 1983, and by Juan Manual Renjifo in February 1984 (Heyer 2005). The geographic coordinates for Campamento Chancos are reported as 3°57'N, 76°44'W (Heyer 2005), and more specific coordinates are provided by Ortega-Andrade (3°57'47"N, 76°44'07"W; 2008). We are confident that these data also correspond

to the specimens of *C. balionota*, as the 460 m elevation reported by Heyer (2005) is identical to that in Lynch and Suarez-Mayorga (2004), and museum numbers of both series of specimens are closely associated (*L. rhodomerus*: ICN 13320–23; *C. balionota*: ICN 13105–13, see Lynch and Ruiz-Carranza 1996). Other amphibian species reported in the literature from Campamento Chancos—presumably collected during the same time-frame based on museum numbers—are endemic to the Chocoan lowlands: *Agalychnis spurrelli*, *Cruziohyla calcarifer*, and *Strabomantis necerus* (Ortega-Andrade 2008; Ospina-Sarria et al. 2015). A search for these latter specimens in the ICN collections database confirmed this connection, and it was also discovered that just one of the *C. balionota* specimens reported by Lynch and Ruiz-Carranza (1996) has been accessioned in the database as of this writing (ICN 13113; <http://biovirtual.unal.edu.co/es/colecciones/search/amphibians/>, Accessed: 8 October 2019); but, notably, our initial search failed to find the specimen because it lacked an identification any more specific than “Centrolenidae.”

Based on current Landsat imagery of the geographic coordinates viewed in Google Earth, specimens of *N. balionotus* from Campamento Chancos were collected in primary Chocoan Tropical Rainforest near the Río Colima, just ~3 km south of the border with the Choco Department. Although relatively little anthropogenic disturbance is evident in the surrounding area, a network of forest clearings along the Río Colima exists just 2 km to the southwest of the collection site. Ospina-Sarria et al. (2015) indicate that the status of amphibian populations from Campamento Chancos are difficult to determine due to the site being in a region with “problems of public order,” which suggests that surveys have likely not been conducted there since 1993 based on the most recent collection date for material from this locality in the ICN database.

In addition to limited data on these specimens, new material for *N. balionotus* from Colombia has not been reported in recent decades. Therefore, it is not currently possible to resolve whether the population from Campamento Chancos is representative of a lineage distinct from those in Ecuador, as Cisneros-Heredia and McDiarmid (2006) suggest. However, it is plausible that this scenario is correct based on available information. In addition to lacking humeral spines, the records from Campamento Chancos are well separated from, and substantially lower in elevation than all other specimens. In fact, the discrepancy in elevational range between Ecuadorian and Colombian populations is so distinct that if Bernal and Lynch (2008) had conducted their analysis of anuran richness and elevational distribution patterns in Ecuador as well, *N. balionotus* would have been categorized in an entirely different assemblage from that in Colombia, and without overlap (i.e., assigned to the Andean assemblage as opposed to the lowland assemblage). Likewise, Ospina-Sarria et al. (2010) would

have excluded the taxon from the list of centrolenids of the Pacific lowlands.

**Conservation status and conclusions.** When the global threatened status for *N. balionotus* was first assessed in 2004, it was determined to be Vulnerable to extinction according to IUCN criteria (VU; Bolívar et al. 2004). That assessment was based in part on the belief that *N. balionotus* was “reasonably common” in Colombia, and with a range that included a relatively large area of potential habitat along the Cordillera Occidental in the departments of Cauca and Nariño (Bolívar et al. 2004). However, and possibly stemming from the aforementioned issues in the literature, it appears the assessment regarded the Campamento Chancos individuals as specimens from “El Tambo” instead of their actual site of collection nearly 200 km further north in the Valle del Cauca Department; and it is also unclear if the assessors realized that no individuals had been reported from Campamento Chancos since 1984. To date, no specimens have been reported from the department of Nariño, Colombia. In Ecuador, the assessment had only accounted for the population from the type locality in Pichincha Province, as the single records from the provinces of Carchi and Cotopaxi were not reported until years after they were collected, i.e., Carchi specimen: collected in 1984, first published by Cisneros-Heredia and Yáñez-Muñoz (2007); Cotopaxi specimen: collected in 2005, first published by Arteaga et al. (2013).

A reassessment of the threatened status of *N. balionotus* has determined it to be Endangered (EN; IUCN SSC Specialist Group 2020). Ecuadorian populations from Mindo and Cabeceras del Río Baboso are likely extirpated based on surveys in those areas since the initial records (Ron et al. 2011, 2015), as well as the Campamento Chancos population in Colombia (see above). The population from El Tambo was also not included in its current distribution (i.e., Presence Uncertain) considering the record is from over 80 years ago (IUCN SSC Specialist Group 2020). Therefore, only the populations from RMR and Río Lomapi are known to represent its current distribution, however the Río Lomapi site does not appear to have been resurveyed since the initial record from 2005. Considering that suitable habitat is likely available between its known localities in both Colombia and Ecuador, it is unclear as to why *N. balionotus* is so uncommon at the sites from which it has been observed, or why it has disappeared from the type locality near Mindo, where habitat modification is not considered to be the cause for its disappearance (Ron et al. 2011). Therefore, it is critical to implement programs to closely monitor and further study the population at RMR. Also, and in light of the substantial rise in mining activity around its range (Roy et al. 2018), efforts to identify additional populations in the region should be prioritized.

If future surveys demonstrate that *N. balionotus* at RMR is indeed restricted to the general areas of observation, and is also sensitive to climatic shifts, the future of this population is likely precarious. That is, all of our records are within the lower half of each slope of the canyon (i.e., closer to the floor of the canyon), which is also the most susceptible area of the canyon to anthropogenic disturbance. If suitable breeding habitat for *N. balionotus* is restricted to streams within old growth forest inside of a relatively narrow elevational band (as our data suggests), its distribution within RMR, and the canyon as a whole, is limited and quickly disappearing on the eastern side of the canyon.

Moreover, and even though a majority of our records are from within the reserve, the future of this population—and those of other threatened taxa at RMR—is uncertain due to mining prospects in the canyon. Attempts to illegally access RMR by a subsidiary of the mining company, BHP Billiton, have continued as of August 2019. A community project initiated by Fundación EcoMinga in the adjacent community of Santa Rosa de Manduriacu aims to engage and connect community members with the unique biological resources and opportunities in the reserve, as well as develop creative enterprises within the community. Fortunately, this project has already helped to encourage community empowerment. During an attempt by the mining company to access RMR in August 2019, community members came together to assert their position that mining personnel will no longer be able to bypass the necessary lines of communication and documentation to gain access to either the reserve or their private property.

Furthermore, BHP Billiton has an established alliance with Conservation International, which is predicated on, “preserving land of high conservation value in key regions where BHP operates” (<https://www.conservation.org/corporate-engagements/bhp-billiton>, Accessed: 18 January 2020). With the addition of *N. balionotus* at RMR, the reserve now contains the only known extant populations of four amphibian species (Lynch et al. 2014; Guayasamin et al. 2019; Reyes-Puig et al. 2020), harbors 15 threatened amphibian and nine reptile species (i.e., IUCN Red List status of VU, EN, or CR; RJM, unpub. data), a new species of *Magnolia* and multiple undescribed orchids (M. Monteros and L. Jost, pers. comm.), a new species and genus of rodent (J. Brito and J. Robayo, pers. comm.), and a number of threatened bird and mammal species (J.M. Loaiza, pers. comm.), including the Critically Endangered Brown-headed Spider Monkey (*Ateles fusciceps*). Considering that these data emphatically demonstrate the exceptional conservation value of this river canyon, we hope to establish a line of communication between our collaborative group of researchers with representatives from both members of the BHP–Conservation International alliance so as to bring attention to this issue and develop a management plan designed to permanently protect the Río Manduriacu

Reserve and the buffer around it. Otherwise, and if parallel circumstances experienced in neighboring regions are an indicator for what is to come for both the local people and the population of *N. balionotus*, their collective futures indeed hang in the balance (Roy et al. 2018).

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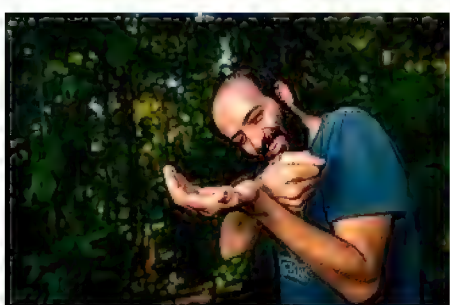
## *Nymphargus balionotus* in Ecuador



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